A Systematic Nomenclature for the Drosophila Ventral Nerve Cord

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Highlights

- A framework defining the anatomy of the adult *Drosophila* ventral nerve cord (VNC)
- A clear and consistent naming scheme for the anatomy of the adult *Drosophila* VNC
- The framework is a tool for integrating past and future work into a common space
- Provides a template that can be adapted to other arthropod nervous systems

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In Brief

The ventral nerve cord (VNC) of *Drosophila* is an important model system for understanding how nervous systems generate locomotion. In this issue of *Neuron*, Court et al. define the structures of the adult VNC to provide an anatomical framework for analyzing the functional organization of the VNC.

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A Systematic Nomenclature for the *Drosophila* Ventral Nerve Cord

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SUMMARY

Drosophila melanogaster is an established model for neuroscience research with relevance in biology and medicine. Until recently, research on the *Drosophila* brain was hindered by the lack of a complete and uniform nomenclature. Recognizing this, Ito et al. (2014) produced an authoritative nomenclature for the adult insect brain, using *Drosophila* as the reference. Here, we extend this nomenclature to the adult thoracic and abdominal neuromeres, the ventral nerve cord (VNC), to provide an anatomical description of this major component of the *Drosophila* nervous system. The VNC is the locus for the reception and integration of sensory information and involved in generating most of the locomotor actions that underlie fly behaviors. The aim is to create a nomenclature, definitions, and spatial boundaries for the *Drosophila* VNC that are consistent with other insects. The work establishes an anatomical framework that provides a powerful tool for analyzing the functional organization of the VNC.

INTRODUCTION

Insects, and *Drosophila melanogaster* in particular, have made huge contributions to neuroscience research (Bellen et al., 2010). The powerful genetic tools and high-resolution neuroanatomy available in flies (Jenett et al., 2012; Scheffer and Meinertzhagen, 2019) and the large number of research groups working on this model will ensure that the fly will remain a powerful tool for analyzing the function and development of complex nervous systems. Here we focus on the organization of an often-overlooked part of the *Drosophila* nervous system, the ventral nerve cord (VNC). The VNC is the insect analog of the vertebrate spinal cord and a significant part of the fly nervous system. The VNC is the locus for the reception and integration of sensory information and is involved in generating most of the locomotor actions that underlie fly behaviors such as walking (Bidaye et al., 2014; Mamiya et al., 2018; Mendes et al., 2013; Tuthill and Wilson, 2016; Wosnitza et al., 2013), grooming (Seeds et al., 2014), jumping (Card and Dickinson, 2008), flying (Dickinson and Muijres, 2016), courtship (Clyne and Miesenböck, 2008), and copulation (Crickmore and Vosshall, 2013; Pavlou et al., 2016). The VNC is, however, not a passive executive center receiving descending signals from the brain; it also sends significant major ascending projections to it (Tsubouchi et al., 2017). While the VNC in *Drosophila* is a complex fusion of all of the sub-gnathal neuromeres, it has a relatively simple and highly ordered structure. From external morphology, it is possible to recognize its constituent segmental neuromeres, the larger of

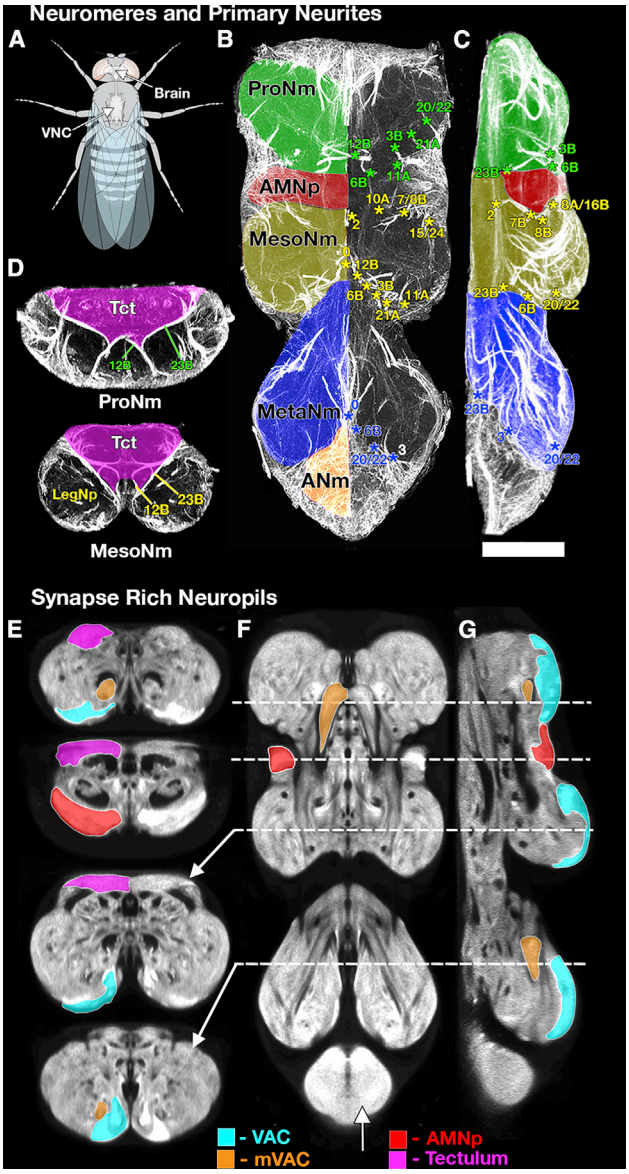

Figure 1. Selected Sections through an Adult VNC Illustrating the Tools Used to Define the Major Structures of the VNC

(A) Schematic of *Drosophila* illustrating the position of the VNC with respect to the body and brain.

(B–D) Neuroglian immunostaining showing neuromeres and Primary Neurite bundles in horizontal (B), lateral (C), and transverse (D) sections to reveal the tracts of the primary neurites of the postembryonic neuronal lineages. The pattern of labeled pathways is highly stereotyped; each pathway corresponds to the primary neurites of neurons derived from a single neuroblast. These tracts provide a robust basis for identifying the key structures of the VNC such as the following: (B and C) the neuromere boundaries (ProNm [green], MesoNm [yellow], MetaNm [blue], and ANm [red]) and (D) the tectulum (magenta—Tct). The numbers refer to specific hemilineage primary neurite bundles, with the color indicating their neuromere of origin.

(E–G) Brp-SNAP labeling (Bogovic et al., 2019) revealing the fine structure of the neuropil shown in transverse (E), horizontal (F), and lateral (G) sections. The *bruchpilot* (Brp) staining reveals characteristic regions of neuropil with high-density staining indicating synapse-rich neuropils. These synapse-rich neuropils can be used to define and segment specific neuropils such as the VAC (cyan), mVAC (orange), AMNp (red), and those of the tectulum (magenta, neck neuropil, wing neuropil, and haltere neuropil). The planes of the sections are indicated by the dotted lines. See also Video S1. A list of the abbreviations is given in Table 1. Scale 50 μm.

which are the three thoracic ones, with the smaller, merged abdominal neuromeres protruding from the posterior end (Figure 1).

As with all arthropods, the neuronal cell bodies of the VNC form an outer cortex with neurons projecting processes centrally to form a dense fibrous central neuropil. The neuropil is stereotyped and highly ordered with functional segregation evident even at the level of the gross anatomy. The VNC is clearly subdivided in the dorso-ventral plane: ventral regions of the thoracic neuropils are innervated by neurons associated with the legs (Merritt and Murphey, 1992), whereas the dorsal neuropils are innervated by neurons associated with the wings and flight (Leise, 1991; Milde et al., 1989; Strausfeld, 1992) with intermediate regions serving to link legs and wing control (Namiki et al., 2018) (Figure 1). At a more detailed level, the neuropils exhibit a fine-grade functional order with modality-specific (Murphey et al., 1989a) and somatotopic (Murphey et al., 1989b) segregation of sensory afferent projections and myotopic organization of motor neuron dendrites (Baek and Mann, 2009; Brierley et al., 2012).

This functional organization of the neuropil provides a rigid anatomical framework against which it is possible to infer the function of neurons simply based on their anatomy. This framework is powerfully informative and an essential tool to analyze how neurons control complex behaviors such as flying, courtship, and walking. Given the fundamental importance of this anatomical order, it is vital that this anatomical framework is robust, with a shared knowledge base to allow researchers to confidently and accurately place neurons within this framework. To achieve this requires a systematic and consistent nomenclature and an anatomical template that precisely defines key anatomical structures, their boundaries, and the terms used to describe them. Recognizing the need for such consistent and robust anatomical framework, a consortium of neurobiologists studying arthropod brains (the insect brain name working group [IBNWG]), was established and produced a comprehensive hierarchical nomenclature system for the insect brain, using *Drosophila melanogaster* as the reference framework (Ito et al., 2014). This effort focused specifically on the brain and the gnathal regions of insects. In this work, we extend the development of a consistent nomenclature and anatomy to the *Drosophila* VNC.

Our work builds on previous descriptions of the *Drosophila* VNC (Power, 1948; Miller and Demerec, 1950; Merritt and Murphey, 1992; Boerner and Duch, 2010). It is also informed by the descriptions of the thoracic and abdominal ganglia of other insects such as grasshopper (Tyrer and Gregory, 1982) and stick insect (Kittmann et al., 1991). These comparative studies also point to clear evolutionary conservation of the basic elements of the *Drosophila* VNC. While these studies, plus many others, have created a rich catalog of anatomical detail, the inconsistent approach to nomenclature and definitions across the field has created ambiguity and confusion. The aim of the *Drosophila*

adult VNC working group (DAVWG) was to create a nomenclature, definitions, and spatial boundaries for the key anatomical entities of the *Drosophila* VNC that are consistent with the nomenclature used to describe the VNC in other insects.

RESULTS

Organization of the Working Group

The initial phase of work followed a similar format to that adopted by the original Insect Brain Name Working Group (IBNWG) to create the nomenclature for the *Drosophila* brain (Ito et al., 2014). We gathered researchers with expertise in the anatomy, development, and physiology of the VNC, hereafter referred to as the *Drosophila* Anatomy of the Ventral nerve cord Working Group (DAVWG) for a workshop at the Janelia Research Campus in October 2013. We discussed a document listing all of the named regions found in the published literature and from the existing *Drosophila* anatomy ontology (Costa et al., 2013), as well as representative anatomical images assembled by authors Court and Shepherd. After systematic review and debate, the participants compiled a working proposal for wider comment. Iterative revisions resulted in the current nomenclature described here.

Establishing the Anatomical Framework

Establishment of a systematic nomenclature requires a clear morphological and spatial definition of all the structures to be named and a standard naming scheme. The neuropil regions of the VNC are typically regarded as being "unstructured" or "tangled," or having a fine, granular appearance in sections with different regions distinguished only by general spatial terms (Merritt and Murphey, 1992). Despite this, fixed landmarks such as longitudinal tracts and commissures can be used to define the structure and organization of different volumes of VNC neuropil (Shepherd et al., 2016).

Developmental origin provides an alternative organizational principle for defining the substructure of the neuropil. Neurons arise from neuroblasts whose first division results in A and B daughter cells. These undergo self-renewing divisions to produce clonal populations referred to as hemilineages. The neurons from a hemilineage tend to share properties, such as neurotransmitter identity and projection pattern—and even function (Harris et al., 2015; Lacin et al., 2019; Shepherd et al., 2019). Shepherd et al. (2016) used the primary projections of neuronal hemilineages to provide an organizational principle for defining the substructure of the neuropil. Although these landmarks may not always correspond to the underlying functional organization, they provide a consistent means of structurally defining neuropil regions.

To provide an initial framework for establishing distinct boundaries within the VNC, we used confocal datasets that reveal various salient features, including tracts and neuropil. The anti-neuroglian antibody (Iwai et al., 1997) (Figures 1B–1D) was used to reveal the primary projections of clonally related neurons in neuroblast (NB) hemilineages (Shepherd et al., 2016). The detailed structure and textural details of the neuropil were based on VNCs labeled to visualize neuropils according to the density of active-zone-specific proteins using anti-*Drosophila* N-cadherin (Shepherd et al., 2016), anti-nc82 (bruchpilot [brp]) (Wagh et al., 2006), or brp-SNAP (Kohl et al., 2014) (Figures 1E–1G and Video S1). For most figures, we have used the high-resolution female VNC template produced by Bogovic et al., 2019, which provides the highest level of resolution and detail currently available. This template can be found at https://www.janelia.org/open-science/jrc-2018-brain-templates. These labels all reveal the fine details of texture and structure in the VNC neuropil, making it possible to distinguish between neuropils that are poor in synapses, such as regions occupied by axons; primary neurites; and glial processes and synapse-rich regions, such as the primary sensory neuropils and the dorsal neuropils associated with the neck, wings, and halteres (Figures 1E–1G). An anti-alpha tubulin antibody (data not shown) was used to reveal fibrous structures such as longitudinal tracts and commissures (Boerner and Duch, 2010). Other images obtained with these labeling methods are available on the Virtual Fly Brain (https://github.com/VirtualFlyBrain/DrosAdultVNSdomains/tree/master/Court2017/template).

Since all of these antibodies are available at low cost through the Developmental Studies Hybridoma Bank created by the NICHD of the NIH and maintained at The University of Iowa, Department of Biology, Iowa City, IA 52242, they can be used by future researchers to counterstain their own samples, identify neuropil regions described in this nomenclature, and computationally register them to our standard reference brains.

The Naming Scheme

All of the anatomical data used in this manuscript can be found on the Virtual Fly Brain GitHub repository https://github.com/VirtualFlyBrain/DrosAdultVNSdomains. All of the text definitions of the structures and synonyms considered in the nomenclature can be found on http://purl.obolibrary.org/obo/fbbt

A key principle was to integrate existing terminology into the standard nomenclature we propose here. We made changes only to remove ambiguity. When multiple names for an anatomical entity were used in the literature, we gave preference to the name that was most commonly used based on citations. While we sought to preserve consistency with terms used for earlier developmental stages and in other insects, we avoided the implication of homology. Most of the naming scheme relies on morphological features rather than functional data, which we incorporate in the definitions when known. We also include a look-up table of synonyms, prior terms, and references.

Abbreviations

We adopted a systematic approach when developing abbreviations for each named anatomical entity based on the following principles: (1) We adopted abbreviations that are unique across the whole CNS, avoiding abbreviations already in use for regions in the brain. (2) We created a system in which related entities would be easily recognizable. (3) We tried to be consistent with nomenclature established for the brain (Ito et al., 2014). The reasoning behind each abbreviation change was recorded and embedded in the definition. When referring to the neuromere and related structures, abbreviations were changed from a single letter or number to "Pro," "Meso," and "Meta." This removed confusion with positional abbreviations such as

posterior or medial. The use of the single letter "N," which is used widely (neuromere, neuropil, nerve, neuron), was reserved for "nerve"; other larger gross anatomy structures differentiated with additional letters (e.g., "Nm" for neuromere and "Np" for neuropil). The letter "C" was used to identify commissures. In cases where multiple abbreviations already exist in the literature for specific structures, the abbreviation that provided the clearest indication with least likelihood of confusion was selected, and additional abbreviations were captured as synonyms. A list of abbreviations is given in Table 1.

Axis Orientation

The general axis of orientation for the VNC is straightforward. The neuroaxis and the body axis are the same, with the prothoracic neuromere being the most anterior and the abdomen (abdominal ganglionic complex) being the most posterior. In the dorsal/ventral plane, the tectulum is dorsal and the leg nerves ventral. The dorsal/ventral axis is also sometimes referred to as superior/inferior, but dorsal and ventral are the preferred terms. The designation of left and right is assigned as if the sample is viewed from above (dorsal). The orientation in all figures is with anterior up for wholemount, lateral and horizontal views and dorsal up for transverse section views.

Definition of the VNC

The VNC is the region of the central nervous system posterior to the brain. It is connected to the brain by descending and ascending neurons that pass through the neck connective. The *Drosophila* VNC is a single consolidated ganglion located in the ventral part of the thorax. This ganglion contains all of the thoracic and abdominal neuromeres (Figure 1) and was called the thoracicoabdominal ganglion by Power (1948); see also synonyms in the supplemental section.

Identifying and Defining the Neuropil Structures in the VNC

Many insects have a ladder-like ventral nervous system composed of physically separated segmental neuromeres connected by longitudinal tracts (connectives), but in *Drosophila*, the thoracic and abdominal neuromeres are fused into a single complex (Niven et al., 2008) located within the thorax (Figure 1A). At the gross anatomical level, the segmental organization of the VNC can be resolved from external morphology. The thoracic neuromeres constitute the bulk of the VNC and are recognizable as three paired enlargements at the anterior of the VNC, corresponding to the prothoracic, mesothoracic, and metathoracic neuromeres (ProNm, MesoNm, and MetaNm, Figures 1B and 1C). At the posterior end is a small, dorsally located mass, the abdominal neuromeres, that is a fusion of all the abdominal neuromeres (ANm, Figure 1B).

Despite the evident external segmental organization, the fusion of multiple neuromeres means that identifying precise neuropil boundaries can be problematic. One of our aims was to define different regions of neuropil and provide landmarks to facilitate consistent identification and nomenclature for future studies. Although the VNC does not have the clearly defined compartmental structure found in the *Drosophila* central brain, it does have a clear architecture of tracts, commissures, and

Table 1. List of the Major Structures and Their Abbreviations

Major Neuromeres and Neuropils	Longitudinal Tracts
Prothoracic neuromere (ProNm), Accessory Mesothoracic neuropil (AMNp), Mesothoracic neuromere (MesoNm), Metathoracic neuromere (MetaNm), Abdominal neuromere (ANm), Tectulum (Tct), Upper tectulum (UTct), Intermediate tectulum (IntTct), Lower tectulum (LTct), Wing tectulum (WTct), Haltere tectulum (HTct), Neck tectulum (NTct), Leg neuropil (LegNp), Intermediate neuropil (IntNp), Ventral Association Centre (VAC), Medial Ventral association centre (mVAC), Intermediate Lateral association centre (iLAC)	Dorsal lateral tract (DLT), Intermediate tract of dorsal cervical fasciculus (ITD), Dorsal lateral tract of ventral cervical fasciculus (DLV), Ventral lateral tract (VLT), Ventral median tract of ventral cervical fasciculus (VTV), Median dorsal abdominal tract (MDT), Ventral cervical fasciculus (VCF), Dorsal cervical fasciculus (DCF), Dorsal median tract (DMT), Ventral ellipse (VE)
Commissures	**Peripheral Nerves**
anterior Anterior Ventral Commissure (aAV), posterior Anterior Ventral Commissure (pAV), Anterior Intermediate Commissure (AI), ventral Anterior Intermediate Commissure (vAI), Anterior Intermediate anterior Commissure (AIa), Anterior Intermediate posterior Commissure (AIp), dorsal Anterior Intermediate Commissure (dAI), anterior Posterior Intermediate Commissure (aPI), posterior Posterior Intermediate Commissure (pPI), dorsal PI Commissure (dPI), Posterior Dorsal Commissures (PD), Commissure of Fine Fibers of the Intermediate Tract of the Dorsal Cervical Fasciculus (CFF), Commissure of Prothoracic Neuromeres (CPN), Dorsal Accessory Commissure of the Mesothoracic Neuromeres (DAM), Ventral Ellipse (VE)	Cervical nerve (CvN), Dorsal prothoracic nerve (DProN), Prosternal nerve (PrN), Prothoracic chordotonal nerve (ProCN), Prothoracic accessory nerve (ProAN), Ventral prothoracic nerve (VProN), Prothoracic leg nerve (ProLN), Anterior dorsal mesothoracic nerve (ADMN), Posterior dorsal mesothoracic nerve (PDMN), Mesothoracic accessory nerve (MesoAN), Mesothoracic leg nerve (MesoLN), Dorsal metathoracic nerve (DMetaN), Metathoracic leg nerve (MetaLN), First abdominal nerve (AbN1), Second abdominal nerve (AbN2), Third abdominal nerve (AbN3), Fourth abdominal nerve (AbN4), Abdominal nerve trunk (AbNT)
Specific Neurons	**Other Structures**
Giant Fiber (GF), Contralateral haltere interneurons (cHIN)	Femoral chordotonal organ (FeCO), Cervical connective (CvC)

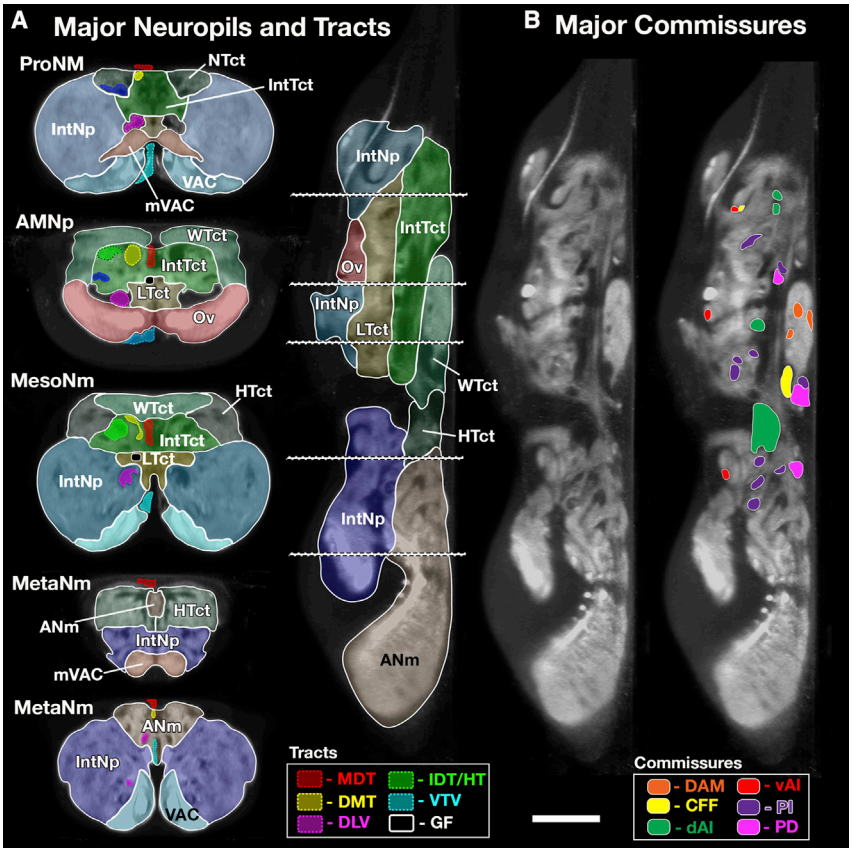

Figure 2. Major Neuropils, Tracts, and Commissures of the VNC

(A) Major Neuropils and Tracts—segmented VNC shown in transverse and lateral sections illustrating the outlines of the major neuropils and longitudinal tracts described in this study. The tectulum domains are shown in different shades of green, and the leg neuropil domains are shown in shades of blue. To further aid visualization, labeled tracts are only shown in the left half of the transverse sections. The plane of the transverse sections is indicated by dotted lines.

(B) The position of the major commissural pathways shown on a lateral section at the midline of the VNC. Tracts derived from the same larval commissure are shown in the same colors. An unlabeled section is provided to show the detail unhindered by labeling. See also Figure S1 and Video S2. A list of the abbreviations is given in Table 1. Scale 50 μm.

axon bundles that provide the basis for defining different regions of neuropil. Cell body positions are not a reliable indicator of the segmental organization of the VNC. There are many examples of cell bodies being passively displaced during neuropil expansion at metamorphosis, resulting in somata being drawn across the midline or pulled into adjacent neuromeres (Shepherd et al., 2019).

Neuromere Boundaries

Although the VNC is a fusion of thoracic and abdominal neuromeres, it is possible to define neuromere boundaries using the scaffold of neuronal fibers revealed by neuroglian expression. The neuroglian positive bundles are the tightly fasciculated primary neurites from individual neuronal lineages, where somata from a lineage remain closely associated with each other. Since each neuromere is founded by a specific set of NBs, the lineage derived neuroglian bundles create a neuromere-specific set of markers, creating a robust framework that clearly outlines the neuropil within each neuromere and thus helps to define the neuropilar boundaries between each neuromere (Figures 1B and 1C). The neuroglian label also provides markers for other structures such as the tectulum (Tct [magenta], Figure 1D) and some commissures (Figure 2) (Shepherd et al., 2016).

Major Subdivisions of the Thoracic Neuropils

While the neuromeres divide the VNC along the anterior-to-posterior axis, there is also specialization on the dorso-ventral axis with a dorsal region called the tectulum (Tct) and a ventral region called leg neuropil (LegNp) (Figure 1D).

The Tectulum (Tct)

The tectulum (Tct) was described by Power (1948) as a discrete dorsal region of the VNC, overlying the mesothoracic neuromere like a saddle and extending from the posterior prothoracic to the anterior metathoracic neuromeres. The neuroglian positive primary neurites provide boundaries that precisely circumscribe the tectulum to define its boundaries (Figure 1D) (Shepherd et al., 2016). Although Power (1948) defined the tectulum as a single neuropil without sub-divisions, the tectulum can be stratified into three layers in the dorsal ventral plane that the working group renamed as upper, intermediate, and lower tectulum (Figure 2A). The lower and intermediate tectulum show no overt signs of segmental barriers and are considered to lack a segmental organization. The upper tectulum, however, does have some segmental specializations and can be segregated on the basis of the synapse rich neuropils revealed by N-Cadherin/bruchpilot expression into three neuromere specific neuropils: neck, wing, and haltere tectulum for the ProNm, MesoNm, and MetaNm neuromeres, respectively (Figures 1B and 2A; Video S2).

The Leg Neuropil

The ventral portion of each thoracic neuropil outside of the tectulum is the leg neuropil (LegNp, see Supplemental Information for details). Unlike the tectulum, the leg neuropils exhibit clear segmental boundaries and, although each thoracic neuromere is slightly different, they all conform to the same organizational principles (Figure 2A; Video S2). The legNps contain the sensory afferent endings of leg sensory neurons, the leg motor neurons, and local interneurons that control leg movement. The leg neuropils are best described in transverse section and can be partitioned into distinct regions along the dorsoventral axis (Figure 2A;

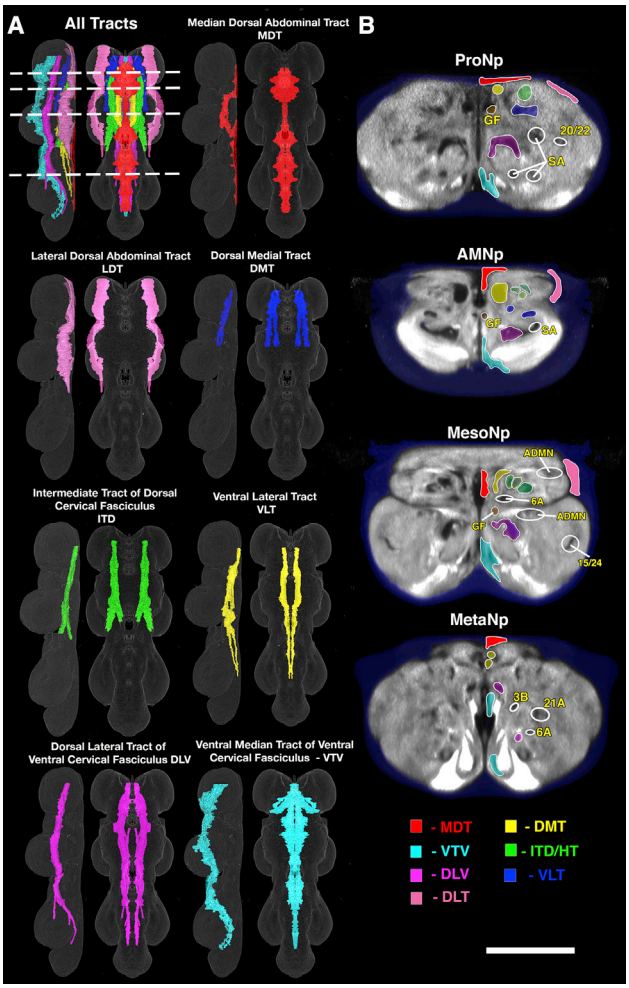

Figure 3. Major Longitudinal Tracts of the VNC

(A) The major tracts of the VNC shown as rendered volumes from lateral and dorsal perspectives.

(B) Transverse section views of the tracts at selected points in the VNC. The areas outlined by white circles identify other key structures (GF, giant fiber; ADMN, sensory afferents entering from the ADMN; SA, sensory afferents entering from the leg nerve; the numbers refer to hemilineage-derived axon fascicles). The planes of section are indicated by dotted lines in (A). See also Video S3. A list of the abbreviations is given in Table 1. Scale (A), 100 μm; (B), 50 μm.

Video S2). The ventralmost layer of leg neuropil, the ventral association center (VAC) (Merritt and Murphey, 1992) is readily distinguishable as synapse rich neuropils (VAC, Figures 1E–1G and 2A; Video S2). The VAC is innervated by sensory afferents from sensory neurons associated with tactile bristles on the leg (Murphey et al., 1989b). Adjacent to the VAC is a paired globular structure, the medial ventral association center (mVAC) (mVAC, Figures 1E–1G and 2A; Video S1). The mVAC is a bilaterally symmetrical neuropil region that can be identified both by its fine textured appearance and as dense synaptic neuropil (Merritt and Murphey, 1992). In *Drosophila*, the mVAC is innervated by a subset of femoral chordotonal organ (FeCO) sensory neurons which form a "club"-shaped projection that terminates in the mVAC (Phillis et al., 1996). The *Drosophila* mVAC is homologous to the mVAC described in locusts and other insects that also receive primary sensory afferents for leg chordotonal organs and is known as "auditory neuropil" (Oshinsky and Hoy, 2002; Römer et al., 1988).

The leg neuropil, between the VAC and the tectulum, is called "intermediate neuropil" (IntNp) because it occupies most of the central third of the dorsoventral area in transverse section (IntNp, Figure 2A; Video S2). The IntNp contains the dendritic branches of the leg motorneurons, premotor interneurons (Shepherd et al., 2019), and sensory afferents from leg campaniform sensilla, hair plates, and the "hook" and "claw" projection types from the FeCO (Mamiya et al., 2018). Like the tectulum, the leg neuropils exhibit clear functional segregation: motor neuron dendrites show clear spatial and functional organization (Maniates-Selvin et al., 2020), and the sensory modalities are partitioned into layers, with proprioception in intermediate neuropil and a somatotopic representation of tactile information in the ventralmost zone (Murphey et al., 1989b; Tsubouchi et al., 2017).

Tracts and Commissures

Building on studies of orthopterous insect ganglia such as the grasshopper (Tyrer and Gregory, 1982), Merritt and Murphey, (1992) and Boerner and Duch (2010) described the stereotyped patterns of longitudinal tracts and commissures in the adult *Drosophila* VNC (Figures 2A, 3, and S1; Video S3). Here we have reviewed these studies and nomenclatures and extended them by providing high resolution volumes for these structures. The nomenclature for the commissures has been redesigned to create a new consistent naming system that reflects the developmental origins of each adult commissure. Truman et al., (2004) showed that the larval VNC has just five commissures per neuromere and that the postembryonic neuronal lineages that cross the midline do so via a specific and invariant commissure (Truman et al., 2004). The five larval commissures split into additional pathways during metamorphosis due to the expansion and extension of the neuropil, so the adult fly has more commissures than the larva (Figures 3 and S1). Using lineage-based markers, Shepherd et al. (2016) linked the larval commissures to their adult counterpart (Power, 1948; Merritt and Murphey, 1992). These lineage-based definitions underlie the proposed nomenclature. Unlike the commissures, the longitudinal tracts were fully described by Power (1948) and Merritt and Murphey (1992) with a largely consistent and widely accepted nomenclature that we have retained.

DISCUSSION

With this nomenclature, we address two primary issues required to create a clearer understanding of the VNC structure and to facilitate dialog and data exchange among neuroscience researchers. The first was to establish a common anatomical framework to precisely define and describe, textually and spatially, the anatomical organization of the VNC. The second was to create a clear and consistent naming scheme for each anatomical entity. The detailed VNC map we provide is essential for integrating past and future work into a common space, thereby contributing to new lines of investigation. In addition, our effort will inform researchers working with other insects, providing them with a

template that can be adapted to their own model organism. Although the nomenclature developed in this project will serve as an initial standard, we acknowledge that to remain useful it must be maintained as a "living" process and evolve as our understanding of the VNC structure and function grows. Future revisions and additions will be required, and there are regions of the neuropil that will benefit from further analysis to provide a clearer breakdown of the substructure. Most notably, the thoracic IntNp, which, although extremely important, still remains a broadly defined region that lacks detailed spatial information, particularly in relation to the spatial organization of sensory neurons and motor neuron dendrites. Such additions and improvements will be handled via the existing online system for posting anatomy ontology suggestions located at https://github.com/FlyBase/Drosophila-anatomy-developmental-ontology/issues and maintained by VirtualFlyBrain.org.

Unlike the brain, the VNC in insects demonstrates significant diversity in its gross organization and structure (Niven et al., 2008). However, there is, a large anatomical literature for several insect groups that exhibit markedly different VNC structures (e.g., grasshoppers, crickets, and moths) that often use the same terms as used for *Drosophila*. The differences among the VNCs of different insects are likely to be largely superficial and simply reflect the pattern of ganglionic fusion. While this fusion does create some anatomical confusion, the basic pattern of tracts and commissures is preserved throughout the insects. Considering the conservation of lineages, tracts, and commissures, insects do exhibit remarkably similar CNS structures despite the distortions imposed by ganglionic fusion. Consequently, it is important not only to have a consistent nomenclature to benefit *Drosophila* researchers but also to develop a nomenclature that can be used as broadly as possible across the insects to create a consistent cross-species terminology. While this would require some work to confirm homology rather than rely on inference from similar structure, extension of a consistent nomenclature to other insects would provide a framework to explore cross-species homologies in the VNC, the evolution of neuronal networks, and the deep evolutionary conservation of the nervous system.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- RESOURCE AVAILABILITY
  - Lead Contact
  - Materials Availability
  - Data and Code Availability
- METHOD DETAILS
  - Anatomical Materials
  - Boundary Drawing and 3D rendering

SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at https://doi.org/10.1016/j.neuron.2020.08.005.

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AUTHOR CONTRIBUTIONS

Conceptualization, W.K., J.W.T., and D.S.; Software, R.C.; Validation, All; Resources, D.S., R.C., and J.B.; Data Curation, R.C., M.C., and J.D.A; Writing—Original Draft, D.S. and R.C.; Writing—Review & Editing, M.C., M.D., R.K.M., A.M.S., J.H.S., T.S., J.C.T., J.W.T., and D.W.W.; Visualization, D.S. and R.C.; Funding Acquisition, All.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Anti-neuroglian	Developmental Studies Hybridoma Bank (Iwai et al., 1997)	Cat.no. BP 104 anti-neuroglian RRID:AB_528402
Anti-*Drosophila* N-cadherin	Developmental Studies Hybridoma Bank	Cat. no. DN-Ex 8 RRID:AB_528121
Deposited Data		
All datasets and segmented domains	https://github.com/VirtualFlyBrain/DrosAdultVNSdomains/tree/master/Court2017/template	This Paper
Experimental Models: Organisms/Strains		
Drosophila melanogaster brp-SNAP transgene	Kohl et al., 2014	Dmel\brp$^{SNAPf\text{-}tag}$
Software and Algorithms		
ITK-SNAP	Yushkevich et al., 2006	http://www.itksnap.org/pmwiki/pmwiki.php RRID:SCR_002010
Fluorender	Scientific Computing and Imaging Institute, University of Utah	https://www.sci.utah.edu/software/fluorender.html RRID:SCR_014303
FIJI	Schindelin et al., 2012	https://fiji.sc/ RRID:SCR_002285
Adobe Premiere	Adobe.com	N/A

RESOURCE AVAILABILITY

Lead Contact

Further information and requests for data and resources should be directed to and will be fulfilled by the Lead Contact, David Shepherd (d.shepherd@bangor.ac.uk).

Materials Availability

This study did not generate any new reagents.

Data and Code Availability

All anatomical datasets and segmented domains have been deposited at Virtual Fly Brain (https://github.com/VirtualFlyBrain/DrosAdultVNSdomains/tree/master/Court2017/template) and are openly available.

METHOD DETAILS

Anatomical Materials

All images are based on previously published data and described methodologies. The anti-neuroglian antibody (Iwai et al., 1997) was used to reveal the primary projections of neuron hemilineages as described by (Shepherd et al., 2016). The structure of the neuropil was revealed using anti-*Drosophila* N-cadherin (Developmental Studies Hybridoma Bank; Cat. no. DN-Ex 8 RRID:AB_528121) as described by (Shepherd et al., 2016), anti-nc82 (Developmental Studies Hybridoma Bank; Cat.no. nc82 anti-Bruchpilot RRID:AB_2314866) as described by (Wagh et al., 2006) and the brp-SNAP transgene (Kohl et al., 2014) as described by (Bogovic et al., 2019).

Boundary Drawing and 3D rendering

Neuropil regions, tracts and commissures were manually painted using ITK-SNAP (Yushkevich et al., 2006, RRID:SCR_002010, http://www.itksnap.org/pmwiki/pmwiki.php) using the adult female VNC template produced by (Bogovic et al., 2019),

(https://www.janelia.org/open-science/jrc-2018-brain-templates). Surface rendered images were generated with Fluorender software (RRID:SCR_014303, https://www.sci.utah.edu/software/fluorender.html). Videos were created with Adobe Premiere from on TIFF stacks created in FIJI (Schindelin et al., 2012) RRID:SCR_002285).